A STOCHASTIC MODEL OF STEM CELL PROLIFERATION, BASED ON THE GROWTH OF SPLEEN COLONY-FORMING CELLS*

BY J. E. TILL, E. A. McCULLOCH, AND L. SIMINOVITCH

DEPARTMENT OF MEDICAL BIOPHYSICS, UNIVERSITY OF TORONTO, AND THE ONTARIO
CANCER INSTITUTE, TORONTO, CANADA

Normal mammalian hemopoietic tissue produces a continuous supply of differentiated blood cells whose functions are essential for life. These functional cells have limited life spans and are incapable of self-renewal. For example, erythrocytes lack nuclei and thus, though admirably adapted to their function of gas transport, are incapable of mitosis. Similarly, lack of proliferative capacity is a feature of mature granulocytes. The replacement of these nondividing cells depends on the activity of less differentiated hemopoietic cells with extensive proliferative capacity. On this basis, blood-forming tissue is considered to consist of three main compartments (Fig. 1). The first, or stem cell compartment, contains cells with extensive proliferative capacity which have the ability to give rise to new stem cells and to differentiated cells. The second, or early differentiated cell compartment, consists of cells with limited proliferative capacity. These give rise to the cells in a third compartment, consisting of specialized cells such as erythrocytes or polymorphonuclear cells, which are adapted to fulfill specific functional roles.[1,2]

Under normal conditions, the numbers of differentiated cells in the blood remain relatively constant; under conditions of stress or increased demand, however, rapid changes in cell number are observed, which are followed by a return to normal levels when the stress is relieved. These features of hemopoiesis imply that the production of differentiated cells is under precise control. Further, since stem cells are responsible for continued cell production, it would appear probable that such stem cells are the sites of action for control mechanisms. Indeed, Lajtha and co-workers have proposed a model for the control of erythropoiesis based on an interaction between the hormone erythropoietin and the members of the stem cell compartment.[3]

Members of the stem cell compartment cannot be recognized with certainty on morphological grounds, and indirect methods have been required for their detection.[4] Recently, however, a technique has been developed which may fulfill the requirements of a direct quantitative test for stem cells. The method is based on the observation that mouse hemopoietic tissue contains a class of cells capable of giving rise to macroscopic colonies in the spleens of irradiated mice (Fig. 2).[5] These colonies have been shown by direct cytological means to derive from single cells.[6] The single cells which give rise to colonies have been given the operational name of "colony-forming cells."

Colony-forming cells are present in hemopoietic tissue in relatively small numbers (approximately one colony-forming cell per 10^4 nucleated cells), and are not known to possess any distinguishing features other than their ability to give rise to spleen colonies. Thus, the properties of colony-forming cells must at present be deduced from an analysis of the colonies to which they give rise. These properties are as follows:
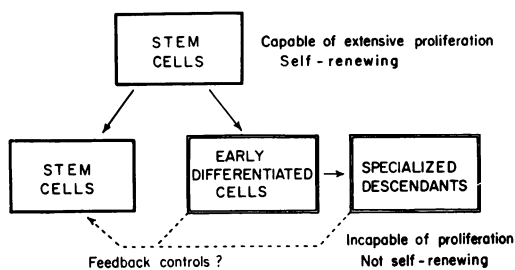
FIG. 1.—A diagrammatic representation of the principal modes of proliferation of blood-forming cells.
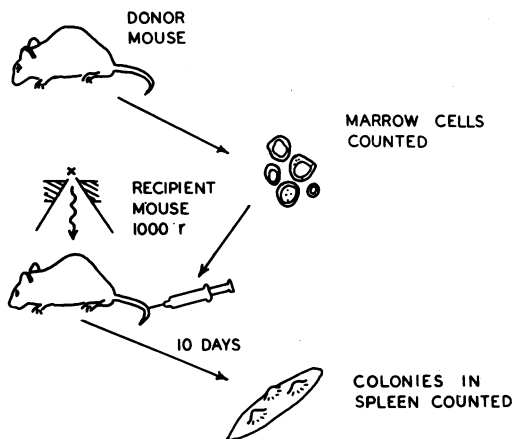

FIG. 2.—Schematic outline of the spleen-colony technique.

First, colony-forming cells possess a capacity for extensive proliferation, since they are able, within 10 days, to give rise to colonies containing in excess of 10^6 cells. Secondly, they are capable of differentiation, since colonies contain large numbers of histologically recognizable differentiated cells.[7] Thirdly, they are capable of self-renewal, since cells which are themselves capable of forming spleen colonies are present within colonies.[8] These properties, proliferative capacity, the related property of self-renewal, and ability to differentiate, are the most important features of members of the stem cell pool; the fact that all three are among the properties of colony-forming cells indicates that these latter cells may be considered as examples of stem cells.

During the development of a colony, a single colony-forming cell gives rise to more than a million progeny, a large majority of which are differentiated as judged by their morphology. Thus, the development of a colony involves processes of differentiation occurring among the progeny from a single cell. Analysis of the cell content of the resulting colony might be expected to cast light on any control mechanisms which act during colony formation. If rigid control mechanisms are operative, acting on cells of a relatively constant genotype, all colony-forming cells might be expected to behave in a similar fashion, and colony formation should be a relatively uniform process, giving rise to colonies with very similar characteristics. Alternatively, if control is lax, colonies with widely differing characteristics might be expected to develop. Results which may bear on this problem are available from experiments in which colonies were analyzed for their content of colony-forming cells.[8] It was found that, while most colonies contained these cells, their distribution among colonies was very heterogeneous, with many colonies containing few colony-forming cells, and a few containing very many. This result suggests that control is lax. In the present paper, an analysis of this variation is presented which indicates that it may be generated by a well-known probabilistic ("stochastic") process, the "birth-and-death" process.[9]

Experimental Procedures.—The assay for colony-forming cells by the enumeration of spleen colonies has been published,[5] and is represented diagrammatically in Figure 2. In brief, a cell suspension is prepared from adult mouse marrow, spleen, or

fetal liver, and an appropriate number of these cells is injected into each of a group of heavily irradiated (900–1000 rads) recipient mice. After 10 days the animals are killed and their spleens fixed in Bouin's solution. This fixation makes the colonies stand out as yellow nodules against the darker background of the splenic tissue. Typical spleens containing colonies are shown in Figure 3. As may be seen from the figure, the colonies are distinct and may be counted with ease.
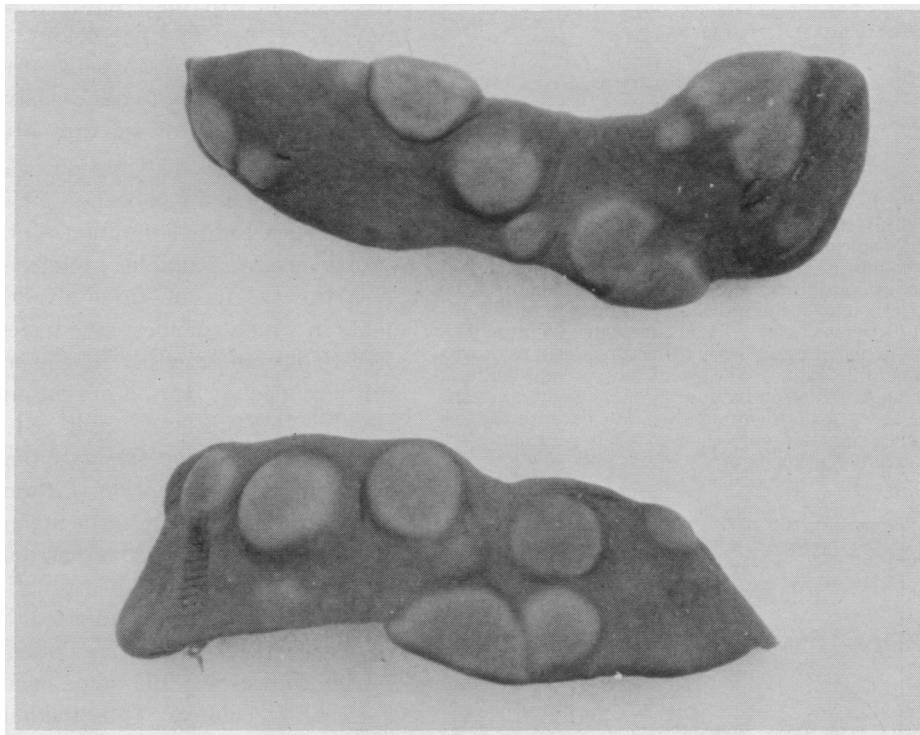

FIG. 3.—Spleens containing 10-day colonies, following fixation in Bouin's solution.

An assay for colony-forming cells within spleen colonies has also been described.[8] In these experiments, spleens containing few (2–4) well-separated colonies were prepared. Each colony was dissected free of spleen, dispersed, and tested for its content of colony-forming cells by counting the number of spleen colonies that developed in the spleens of irradiated mice injected with the cell suspension derived from the colony. It should be noted that an unavoidable dilution of the cell suspension occurs when it is injected into the irradiated recipient, since only a fraction, f, of the colony-forming cells injected reach the spleen and there form colonies. The cells that form spleen colonies are referred to as "colony-forming units" (CFU). For marrow cells, f has been found to be 0.17 ± 0.02.[8] On this basis, the experimentally determined value of CFU per colony must be multiplied by $1/0.17$ in order to obtain the number of colony-forming cells per colony. By this procedure, the content of colony-forming cells in colonies after 10–14 days of growth was determined. The detailed experimental procedures and the results obtained have been published.[8]
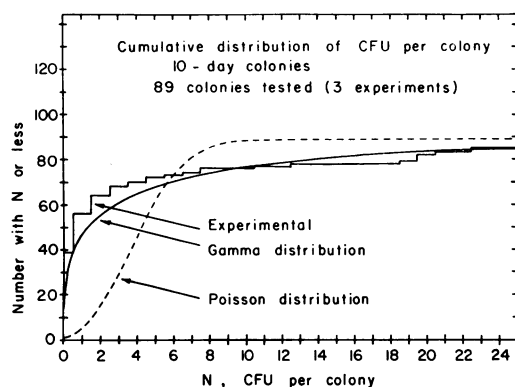
FIG. 4.—Results of a determination of the number of colony-forming units (CFU) present in individual 10-day spleen colonies. Abscissa: number of CFU per colony (N). Ordinate: cumulative number of colonies containing N or less CFU per colony. To obtain colony-forming cells per colony, multiply N by 1/0.17 (see text). The dashed curve represents a Poisson distribution having the same mean as the experimental data. The solid curve represents a gamma distribution having the same mean and variance as the experimental data. The data for the few colonies showing more than 25 CFU per colony are not included in the figure.
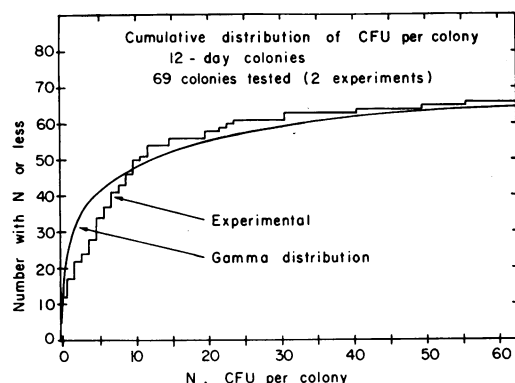

FIG. 5.—Cumulative distribution of CFU per colony in individual 12-day spleen colonies (cf. Fig. 4). The smooth curve represents a gamma distribution having the same mean and variance as the experimental data.

Distribution of Colony-forming Cells among Colonies.—The results of experiments[8] in which the number of colony-forming cells in individual colonies was determined are given in the form of histograms in Figures 4 and 5. In these figures, the number, N, of CFU per colony is plotted on the abscissa against the cumulative number of colonies having N or less CFU on the ordinate. This cumulative distribution was used as it tends to smooth out the data when a limited number of observations are available. Figure 4 gives the results of three experiments in which colonies were tested after 10 days of growth. The mean and variance of the observations are 4.5 CFU/colony and 81.4 (CFU/colony),[2] respectively, so that the coefficient of variation (ratio of the standard deviation to the mean) is 2.0. This heterogeneous distribution, in which the variance greatly exceeds the mean, is compared in the figure with a cumulative Poisson distribution having the same mean of 4.5 CFU/colony. It is evident from the figure, and from the observation that the mean and variance of the experimental data are unequal, that the experimental results are not consistent with a Poisson distribution. This indicates that the variation from colony to colony is greater than might be expected from sampling errors alone.

In the same figure, another type of distribution is also shown. This is a gamma distribution, which is a continuous distribution for which the variance may exceed the mean.[10] The solid curve shown in the figure was constructed from tabulated values[11] of the cumulative gamma distribution, assuming a mean and variance for the distribution equivalent to the observed values given above. A test of goodness of fit indicated that the experimental data do not deviate significantly from the gamma distribution ($P > 0.1$).

In order to test the possibility that the distribution of colony-forming cells

among colonies might be a function of the duration of growth of the colonies, measurements were made after different intervals of growth. In Figure 5 the results of two experiments in which the 69 colonies were tested after 12 days of growth are plotted in the same way as in Figure 4. The experimentally determined mean and variance for these experiments were 13.8 CFU/colony and 737 (CFU/colony).[2] The cumulative gamma distribution with the same mean and variance is shown as a smooth curve. It is apparent that the mean number of colony-forming cells per colony has increased with increased duration of growth. However, the relative heterogeneity has not changed since the coefficient of variation for the 12-day results is 2.0, i.e., the same as that for the 10-day data. Though the fit of the 12-day data to the gamma distribution is less good ($P < 0.01$), there is no doubt that the data may be much more closely approximated by a gamma distribution than by a Poisson distribution. Similar results were obtained for colonies measured after 14 days of growth.

The Birth-and-Death Process.—There are a number of ways in which such heterogeneous distributions may arise (cf. Anscombe[12]). One of these, which is of particular interest from the viewpoint of this paper, has been studied in considerable detail in connection with the theory of cosmic ray showers,[13] and has also been applied to the growth of biological populations (for a recent review, see Bharucha-Reid[14]). The mechanism is the so-called "birth-and-death" process. The process is operative when an entity, for example, a single cell, may either give rise to progeny like itself ("birth"), or be removed in some way ("death"), and these two events occur in a random fashion. The following considerations suggest that a "birth-and-death" process may be operative during the growth of spleen colonies. During the development of a colony from a single cell, a small number of new colony-forming cells and a large number of cells without colony-forming capacity are produced. The latter may be assumed to be the differentiated cells within colonies. Thus, many of the progeny of colony-forming cells lose the capacity to form colonies as they acquire the capacity to fulfill differentiated functions. This loss of colony-forming ability with differentiation may be considered as a "death" process, and contrasts with the self-renewal of colony-forming cells, which is a "birth" process. The two alternative fates postulated for colony-forming cells are illustrated schematically in Figure 6. A third alternative also deserves mention. It is, of course, possible that upon division of a colony-forming cell, one new colony-forming cell and one differentiated cell are produced. However, unless individual divisions can be observed, this process cannot be distinguished from a "birth" followed by a "death." Thus, this possibility need not be treated separately, but can be taken into account by a suitable adjustment of the probabilities per division that a birth or death process occurs. These probabilities are designated p_2 and p_0, respectively, in Figure 6.

It thus appears reasonable on biological grounds to assume that a "birth-and-death" process may be operative during colony formation. If birth or death processes occur at random in each colony-forming cell, governed only by p_2 or p_0, and independently of the previous history of the cells, one may readily set up a model of the process of colony-formation by the use of probabilistic methods.[9, 13, 14] If the assumption of randomness is valid, the results obtained in the model must be consistent with the experimentally determined distribution.

Analyses of the birth-and-death process involving various assumptions concerning the distribution of generation times[9, 15] and the time-dependence of the birth and death probabilities[16] have been made. Unfortunately, explicit solutions for the cell number distributions have been obtained only in the case where the distribution of generation times is a negative exponential.[17] Though this is a rather unrealistic distribution, we can tentatively assume that the form obtained in this case for the cell number distributions (a negative binomial distribution[9]) will be applicable to the situation being studied experimentally. From this point of view, it is of particular interest that the limiting form of the negative binomial distribution is the gamma distribution.[10] Thus, it appears reasonable to assume that, if the random birth-and-death hypothesis is valid, one might expect the distribution of colony-forming cells per colony to be approximated by a gamma distribution regardless of the time dependence of the birth and death probabilities and regardless of the distribution of generation times.

A test of this assumption was made by the use of the Monte Carlo method.[14] In this method, the choice between occurrence or nonoccurrence of a random event is made by drawing a number from a table of random digits.[18] Consider as an example the case in which the birth and death probabilities, p_2 and p_0, are arbitrarily set equal to 0.6 and 0.4, respectively. Let the six digits, 0, 1, 2, 3, 4, and 5 signify a "birth," and the four digits 6, 7, 8, and 9 signify a "death." If the first random number to be drawn was a 5, then the model would show a birth, or an increase from one cell to two cells. Each subsequent choice of a number would determine the fate of a cell in a similar manner. Two examples are shown diagrammatically in Figure 7 where the calculation has been carried through six generations.

More extensive case histories of the type shown in Figure 7 were constructed using an IBM 7090 digital computer. The results of 71 case histories each carried
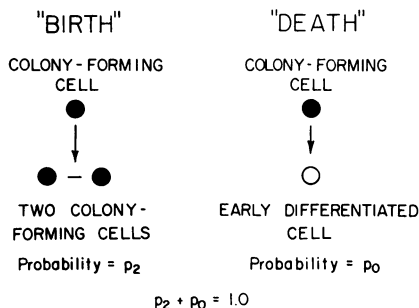

FIG. 6.—Illustration of the "birth " and "death" processes which may be assumed to act during the growth of spleen colonies.
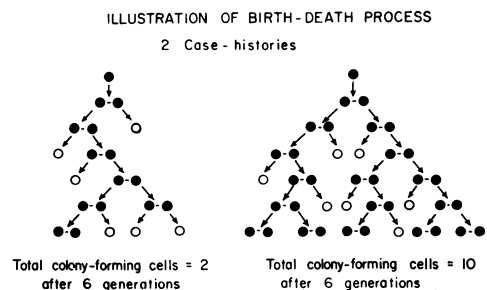
FIG. 7.—Diagrammatic illustration of two Monte Carlo case histories carried through six generations.

through 20 generations are shown in Figure 8. The results are plotted using the same cumulative method as in the experimental histograms of Figures 4 and 5. The smooth curve shown in the figure is a gamma distribution having the same mean and variance as the distribution obtained by the Monte Carlo method. It may be seen that the gamma distribution provides a good fit to the points derived from the Monte Carlo calculations.

The Monte Carlo calculations shown in Figure 8 were made with the assumption of fixed birth and death probabilities and a fixed generation time, with birth or death occurring only at the end of each generation time interval. The form of the cell number distribution obtained in this case was similar to that expected for the case where the distribution of generation times is a negative exponential, which supports the assumption that the birth-and-death process generates a distribution which may be adequately approximated by a gamma distribution, regardless of the distribution of generation times.
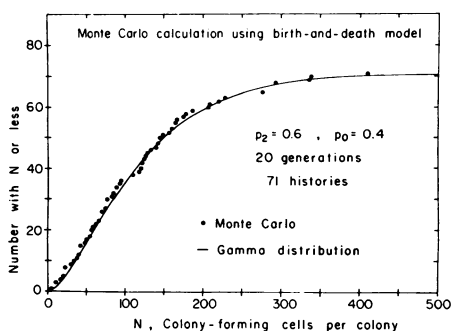

FIG. 8.—The results of Monte Carlo calculations carried out under the assumption of constant birth and death probabilities and fixed cell generation time, with birth or death occurring only at the end of each generation time interval. The smooth curve represents a gamma distribution having the same mean and variance as the calculated distribution.

Analyses such as those outlined above also predict a discrepancy between the doubling time and the mean generation time of colony-forming cells. The former, the doubling time, is the time required for the mean number of colony-forming cells to increase by a factor of two; the latter, the mean generation time, is the mean time interval between successive cell divisions. Since only a portion of the completed cell cycles of colony-forming cells result in an increase in number of these cells, the doubling time must be longer than the generation time. Preliminary experiments confirm this prediction, and add support to the view that the growth of spleen colonies involves a birth-and-death process.

Discussion.—The model of the proliferation of cells during the growth of spleen colonies which has been described in this paper has the following features:

First, every colony-forming cell may follow one of two pathways. On the one hand, the cell may divide and produce two new cells with the capacity to form colonies. On the other hand, the cell may differentiate, and in so doing, lose the capacity for colony formation, although it may retain the ability to undergo several divisions, producing a number of fully differentiated descendants. The second feature of the model is that these two processes, the one a "birth" process, the other a "death" process, occur at random in the population of colony-forming cells. This implies that individual cells within the population are not closely regulated. The question arises as to how this lax regulation can be reconciled with the orderly behavior of normal hemopoietic tissue. An analogy with the decay of radioactive nuclides may be helpful in this regard. If one studies a large number of radioactive atoms, one sees a very regular pattern of decay, following an exponential law. However, if one studies individual atoms, they are found to decay in an unpredictable fashion, at random. It appears possible that our studies of the progeny of single cells display the random feature of hemopoietic function, while a study of large populations of cells reveals the orderly behavior of the whole system. From this point of view, it is the population as a whole that is regulated rather than individual cells, and it is suggested that control mechanisms act by varying the "birth" and "death" probabilities.

At present, the evidence in support of a probabilistic control mechanism is based entirely on the results of one type of experiment, and alternative experimental approaches will be required in order to test the hypothesis. This work is under way. If the probabilistic view is found to be correct, it will still be necessary to determine what kind of control mechanisms could act to alter the probabilities and to define their modes of action.

[1] Cronkite, E. P., T. M. Fliedner, V. P. Bond, and J. S. Robertson, in *The Kinetics of Cellular Proliferation*, ed. F. Stohlman, Jr. (New York: Grune and Stratton, 1959), p. 1.

[2] Patt, H. M., and H. Quastler, *Phys. Rev.*, **43**, 357 (1963).

[3] Lajtha, L. G., R. Oliver, and C. W. Gurney, *Brit. J. Haematol.*, **8**, 442 (1962).

[4] Gurney, C. W., L. G. Lajtha, and R. Oliver, *Brit. J. Haematol.*, **8**, 461 (1962).

[5] Till, J. E., and E. A. McCulloch, *Radiation Res.*, **14**, 213 (1961).

[6] Becker, A. J., E. A. McCulloch, and J. E. Till, *Nature*, **197**, 452 (1963).

[7] McCulloch, E. A., *Rev. Franc. Études Clin. Biol.*, **8**, 15 (1963).

[8] Siminovitch, L., E. A. McCulloch, and J. E. Till, *J. Cellular Comp. Physiol.*, in press.

[9] Feller, W., in *An Introduction to Probability Theory and Its Applications* (New York: Wiley and Sons, 1957), vol. 1, 2nd ed.

[10] Parzen, E., in *Modern Probability Theory and Its Applications* (New York: Wiley and Sons, 1960), p. 260.

[11] Pearson, K, *Tables of the Incomplete Gamma-Function* (Cambridge, 1957).

[12] Anscombe, F. J., *Biometrika*, **37**, 358 (1950).

[13] Arley, N., *On the Theory of Stochastic Processes and Their Application to the Theory of Cosmic Radiation* (New York: Wiley and Sons, 1943).

[14] Bharucha-Reid, A. T., *Elements of the Theory of Markov Processes and Their Applications* (New York: McGraw-Hill, 1960).

[15] Waugh, W. A. O'N., *Biometrika*, **42**, 291 (1955).

[16] Kendall, D. G., *Ann. Math. Statist.*, **19**, 1 (1948).

[17] Harris, T. E., in *The Kinetics of Cellular Proliferation.*, ed. F. Stohlman, Jr. (New York: Grune and Stratton, 1959), p. 368.

[18] The RAND Corporation, *A Million Random Digits with 100,000 Normal Deviates* (Glencoe, Illinois: The Free Press, 1955).

CLARIFICATION OF NATIVE DNA SOLUTIONS BY FILTRATION*

BY ALVIN I. KRASNA† AND JERRY A. HARPST‡

DEPARMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, SAN DIEGO, LA JOLLA

The elimination of dust from solutions of nucleic acids has been a major barrier in obtaining reliable low-angle light-scattering data, which are essential for determining accurate molecular weights.[1] Most workers have relied upon extensive centrifugation with extremely careful handling of the resulting solutions. This procedure is time-consuming, and the results are not entirely satisfactory. Recently, Froelich, Strazielle, Bernardi, and Benoit[2] have recommended shaking the